PCT/EP

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>30 September 1997 (30.09.97) | |
| **International application No.**<br>PCT/EP97/00994 | **Applicant's or agent's file reference**<br>JV/P31395 |
| **International filing date** (day/month/year)<br>26 February 1997 (26.02.97) | **Priority date** (day/month/year)<br>02 March 1996 (02.03.96) |

**Applicant**

MOSSAKOWSKA, Danuta, Ewa, Irena et al

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   16 September 1997 (16.09.97)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No.: (41-22) 740.14.35

Authorized officer

F. Gateau

Telephone No.: (41-22) 338.83.38

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P. ENT COOPERATION TREA

# PCT

## NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

REDDIE & GROSE
16 Theobalds Road
London WC1X 8PL
ROYAUME-UNI

**Date of mailing** (day/month/year)
09 February 1998 (09.02.98)

**Applicant's or agent's file reference**
39156/JMD

**IMPORTANT NOTIFICATION**

**International application No.**
PCT/EP97/00994

**International filing date** (day/month/year)
26 February 1997 (26.02.97)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| SMITH, Richard, Anthony, Godwin<br>SmithKline Beecham Pharmaceuticals<br>New Frontiers Science Park South<br>Third Avenue<br>Harlow<br>Essex CM19 5AW<br>United Kingdom | GB | GB |
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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

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| | Teleprinter No. | |

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

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[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
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**The International Bureau of WIPO**
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**1211 Geneva 20, Switzerland**

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Authorized officer
F. Gateau

Telephone No.: (41-22) 338.83.38

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# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

To:

REDDIE & GROSE
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### NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

**Date of mailing** (day/month/year)
27 November 1997 (27.11.97)

**Applicant's or agent's file reference**
39156/JMD

**IMPORTANT NOTIFICATION**

**International application No.**
PCT/EP97/00994

**International filing date** (day/month/year)
26 February 1997 (26.02.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
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State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
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[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No.: (41-22) 740.14.35

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F. Gateau

Telephone No.: (41-22) 338.83.38

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# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

**NOTIFICATION OF THE RECORDING OF A CHANGE**

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**Date of mailing** (day/month/year)
27 November 1997 (27.11.97)

**Applicant's or agent's file reference**
39156/JMD

**IMPORTANT NOTIFICATION**

**International application No.**
PCT/EP97/00994

**International filing date** (day/month/year)
26 February 1997 (26.02.97)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

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State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

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State of Nationality

State of Residence

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(01223) 360280

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[X] other: VALENTINE, Jill, Barbara

**The International Bureau of WIPO**
**34, chemin des Colombettes**
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PATENT COOPERATION TREATY

# PCT

09/142043

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| JV/P31395 | | |
| International application No. | International filing date (day/month/year) | (Earliest) Priority Date (day/month/year) |
| PCT/EP 97/00994 | 26/02/1997 | 02/03/1996 |

Applicant

SMITHKLINE BEECHAM P.L.C. et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant.
   - [X] the text has been established by this Authority to read as follows:

   FRAGMENTS OF CR1 AND THEIR USE

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

# INTERNATIONAL SEARCH REPORT

[redacted]ational application No.
PCT/EP 97/00994

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 23, 25-30
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 23, 25-30 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The use of expressions like "altered (to what ?) and "chemically reactive amino acids" (under which conditions ?) in claim 5 renders this claim unclear under Art. 6 PCT. Claims searched completely: 1-4,6-36
Claim not searched: 5

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/00994

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C12N15/12 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 94 00571 A (SMITHKLINE BEECHAM PLC ;SMITH RICHARD ANTHONY GODWIN (GB); DODD IA) 6 January 1994 cited in the application see the whole document --- | 1-4,6-36 |
| A | EP 0 512 733 A (UNIV WASHINGTON) 11 November 1992 see the whole document --- | 1-4,6-36 |
| A | WO 91 05047 A (UNIV JOHNS HOPKINS ;BRIGHAM & WOMENS HOSPITAL (US); T CELL SCIENCE) 18 April 1991 cited in the application see the whole document --- | 1-4,6-36 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 July 1997 | 07.08.97 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016 | Authorized officer<br>Groenendijk, M |

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Application No
PCT/EP 97/00994

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 94 16719 A (SMITHKLINE BEECHAM PLC ;MOSSAKOWSKA DANUTA EWA IRENA (GB); SMITH R) 4 August 1994 see the whole document | 31 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/00994

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9400571 A | 06-01-94 | EP 0649468 A | 26-04-95 |
| | | JP 7508516 T | 21-09-95 |
| EP 0512733 A | 11-11-92 | AU 657751 B | 23-03-95 |
| | | AU 1590292 A | 05-11-92 |
| | | CA 2067653 A | 04-11-92 |
| | | JP 6016696 A | 25-01-94 |
| | | US 5545619 A | 13-08-96 |
| WO 9105047 A | 18-04-91 | AU 656312 B | 02-02-95 |
| | | CA 2067744 A | 27-03-91 |
| | | CN 1053265 A | 24-07-91 |
| | | EP 0502892 A | 16-09-92 |
| | | GR 90100716 A | 20-01-92 |
| | | JP 5504053 T | 01-07-93 |
| | | US 5472939 A | 05-12-95 |
| | | US 5256642 A | 26-10-93 |
| WO 9416719 A | 04-08-94 | AU 5863694 A | 15-08-94 |
| | | CA 2153797 A | 04-08-94 |
| | | CN 1118141 A | 06-03-96 |
| | | EP 0680332 A | 08-11-95 |
| | | JP 8505867 T | 25-06-96 |
| | | NZ 259737 A | 24-04-97 |
| | | ZA 9400398 A | 15-11-94 |

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(22) International Filing Date: 26 February 1997 (26.02.97)

(30) Priority Data: 9604518.2 — 2 March 1996 (02.03.96) — GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: FRAGMENTS OF CR1 AND THEIR USE

(57) Abstract

A polypeptide comprising a portion of the sequence of the general formula (I): CNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSG, of 6 to 23 amino acids in length and comprising sequence a) and/or b): a) GGRKVF, b) FELVGEPSIY multimeric and chimaeric derivatives, pharmaceutical compositions containing them and their use in therapy.

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| UZ | Uzbekistan |
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# FRAGMENTS OF CR1 AND THEIR USE

The present invention relates to polypeptides and their use in the diagnosis and therapy of disorders involving complement activity and various inflammatory and immune disorders.

Constituting about 10% of the globulins in normal serum, the complement system is composed of many different proteins that are important in the immune system's response to foreign antigens. The complement system becomes activated when its primary components are cleaved and the products alone or with other proteins, activate additional complement proteins resulting in a proteolytic cascade. Activation of the complement system leads to a variety of responses including increased vascular permeability, chemotaxis of phagocytic cells, activation of inflammatory cells, opsonization of foreign particles, direct killing of cells and tissue damage. Activation of the complement system may be triggered by antigen-antibody complexes (the classical pathway) or, for example, by lipopolysaccharides present in cell walls of pathogenic bacteria (the alternative pathway).

Complement activation (CA) is known to occur in a wide variety of acute inflammatory processes particularly those associated with ischaemia and reperfusion injury (Rossen et al., 1985 Circ. Res., 57, 119,; Morgan B.P., 1990 The biological effects of complement activation. In *'Complement, Clinical Aspects and Relevance to Disease'*. Academic Press. London.)

It is generally accepted that at least some of the components of the classical complement cascade can be detected by immunohistochemical methods in close association with senile plaques in AD brain (Eikelenboom et al., 1994, Neuroscience, 59, 561-568). There is good evidence for the involvement of C1, C3 and C4, but evidence for the presence of the C5-C9 membrane-attack complex (MAC) is not yet evident (Veerhuis et al, 1995, Vichows Arch. 426, 603-610). Cells of the CNS have been shown to synthesise complement components (for review see Barnum, 1995 Crit. Rev. Oral. Biol. Med 6, 132-146), and production of C3 is enhanced in response to incubation with bA4 peptide (Haga et al., 1993 Brain Res., 601, 88-94). Thus complement can be induced locally in the brain itself and is not necessarily derived solely from the plasma compartment.

Of particular interest is the fact that the bA4 peptide has been found to bind directly to the initial component of the complement cascade (C1q) and to initiate the whole of the classical complement system *in vitro* (including MAC) by an antibody-independent mechanism (Rogers et al., 1992, Proc. Nat. Acad. Sci. USA., 89, 10016-10020.; Jianh et al., 1994, J. Immunol., 152, 5050-5059). This interaction appears to involve region 6-16 of βA4 and 14-26 of the collagen-like tail region of the C1q A

chain. The latter site is separate from the IgG-immune complex binding site located on the globular head domain of C1q. There is some evidence that fibrillar bA4 binds with higher affinity to C1q than monomeric peptide, potentially providing a rational basis for activation of complement in the disease process (Jiang et al., 1994, J. Immunol., 152, 5050-5059; Snyder et al., 1994, Exp. Neurol., 128, 136-142).

Complement receptor type 1 (CR1) has been shown to be present on the membranes of erythrocytes, monocytes/macrophages, granulocytes, B cells, some T cells, splenic follicular dendritic cells, and glomerular podocytes. CR1 binds to the complement components C3b and C4b and has also been referred to as the C3b/C4b receptor. The structural organisation and primary sequence of one allotype of CR1 is known (Klickstein *et al.*, 1987, J. Exp. Med. 165:1095-1112, Klickstein *et al.*, 1988, J. Exp. Med. 168:1699-1717; Hourcade *et al.*, 1988, J. Exp. Med. 168:1255-1270, WO 89/09220, WO 91/05047). It is composed of 30 short consensus repeats (SCRs) that each contain around 60-70 amino acids. In each SCR, around 29 of the average 65 amino acids are conserved. Each SCR has been proposed to form a three dimensional triple loop structure through disulphide linkages with the third and first and the fourth and second half-cystines in disulphide bonds. CR1 is further arranged as 4 long homologous repeats (LHRs) of 7 SCRs each. Following a leader sequence, the CR1 molecule consists of the N-terminal LHR-A, the next two repeats, LHR-B and LHR-C, and the most C-terminal LHR-D followed by 2 additional SCRs, a 25 residue putative transmembrane region and a 43 residue cytoplasmic tail.

Based on the mature CR1 molecule having a predicted N-terminal glutamine residue, hereinafter designated as residue 1, the first four SCR domains of LHR-A are defined herein as consisting of residues 2-58, 63-120, 125-191 and 197-252, respectively, of mature CR1.

Hourcade *et al.*, 1988, J. Exp. Med. 168:1255-1270 observed an alternative polyadenylation site in the human CR1 transcriptional unit that was predicted to produce a secreted form of CR1. The mRNA encoded by this truncated sequence comprises the first 8.5 SCRs of CR1, and encodes a protein of about 80 kDa which was proposed to include the C4b binding domain. When a cDNA corresponding to this truncated sequence was transfected into COS cells and expressed, it demonstrated the expected C4b binding activity but did not bind to C3b (Krych *et al.*, 1989, FASEB J. 3:A368; Krych *et al.* Proc. Nat. Acad. Sci. 1991, 88, 4353-7). Krych *et al.*, also observed a mRNA similar to the predicted one in several human cell lines and postulated that such a truncated soluble form of CR1 with C4b binding activity may be synthesised in humans.

In addition, Makrides *et al.* (1992, J. Biol. Chem. 267 (34) 24754-61) have expressed SCR 1 + 2 and 1 + 2 + 3 + 4 of LHR-A as membrane-attached proteins in CHO cells.

Several soluble fragments of CR1 have also been generated via recombinant DNA procedures by eliminating the transmembrane region from the DNAs being expressed (WO 89/09220, WO 91/05047). The soluble CR1 fragments were functionally active, bound C3b and/or C4b and demonstrated Factor I cofactor activity depending upon the regions they contained. Such constructs inhibited *in vitro* complement-related functions such as neutrophil oxidative burst, complement mediated hemolysis, and C3a and C5a production. A particular soluble construct, sCR1/pBSCR1c, also demonstrated *in vivo* activity in a reversed passive Arthus reaction (WO 89/09220, WO 91/05047; Yeh *et al.*, 1991, J. Immunol. 146:250), suppressed post-ischemic myocardial inflammation and necrosis (WO 89/09220, WO 91/05047; Weisman *et al.*, Science, 1990, 249:146-1511; Dupe, R. *et al.* Thrombosis & Haemostasis (1991) 65(6) 695.) and extended survival rates following transplantation (Pruitt & Bollinger, 1991, J. Surg. Res 50:350; Pruitt *et al.*, 1991 Transplantation 52; 868). Furthermore, co-formulation of sCR1/pBSCR1c with p-anisoylated human plasminogen-streptokinase-activator complex (APSAC) resulted in similar anti-haemolytic activity as sCR1 alone, indicating that the combination of the complement inhibitor sCR1 with a thrombolytic agent was feasible (WO 91/05047).

In a model of antibody-mediated demyelinating experimental allergic encephalomyelitis (ADEAE), systemic inhibition of CA using sCR1 over 6 days, produced improvements in clinical score and blocked CNS inflammation, demyelination and deposition of complement components (Piddlesden et al., 1994, J. Immunol. 152, 5477). ADEAE can be regarded as a model of acute relapse in multiple sclerosis (MS) and these striking results suggested possible applications for sCR1 in MS therapy despite the high molecular weight (245 kilodaltons) of this agent.

In a rat model of traumatic brain injury, complement inhibitor sCR1 (BRL55730) was shown to reduce myeloperoxidase activity (an indicator of neutrophil accumulation) following traumatic injury (Kaczorowska et al, 1995, J. Cerebral Blood Flow and Metabolism, 15, 860-864). This is suggested as demonstrating that complement activation is involved in the local inflammatory response.

Soluble polypeptides corresponding to part of CR1 having functional complement inhibitory, including anti-haemolytic, activity have been described in WO94/00571 comprising, in sequence, one to four short consensus repeats (SCR)

selected from SCR 1, 2, 3 and 4 of long homologous repeat A (LHR-A) s the only structurally and functionally intact SCR domains of CR1 and including at least SCR3.

According to the present invention there is provided a polypeptide comprising a portion of the sequence of the general formula (I):

CNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSG (1)

of 6 to 23 amino acids in length and comprising sequence a) and/or b):

a) GGRKVF

b) FELVGEPSIY

The peptides of the invention are derived from the region of SCR3 of human CR1 between amino acids C154 to G186.

It is to be understood that variations in the amino acid sequence of the polypeptide of the invention by way of addition, deletion or conservative substitution of residues, including allelic variations, in which the biological activity of the polypeptide is retained, are encompassed by the invention. Conservative substitution is understood to mean the retention of the charge, hydrophobicity/hydrophilicity and size characteristics of the amino acid side chain, for example arginine replaced by histidine or lysine.

The polypeptide may be modified to have cysteine residues at the C and N termini to provide a molecule capable of forming a cyclic molecule bridged by a disulphide bond. The peptide may also be altered at specific amino acids to remove chemically reactive amino acids such as cysteine, or replace such amino acids by conservative substitutions such as serine.

The polypeptide may have chemically reactive amino acids such as cysteine, lysine or glutamic acid at the N or C-terminal ends optionally further derivatised or derivatisable to provide a route for chemical linkage to other peptides or chemicals. Preferably, the terminal amino acid is cysteine and a derivative is S- (2-pyridyl) dithio.

Enhanced activity may be achieved by forming multimerised polypeptides. According to the present invention there is provided a multimeric polypeptide comprising two or more, for example two to eight, polypeptides of the invention, linked to a core structure which may be a core peptide or multifunctional molecule. The core peptide is preferably a lysine derivative such as the 'MAP' peptide ( Posnett, D.N. &Tam, J.P, Methods in Enzymology, 1989, 178, 739-746) exemplified by $(lys)_4(lys)_2$ lys ala in which the first lysine has two further lysines linked to both alpha and episilon amino groups and the second two lysines each have two further lysines thus giving a branched (dendritic) polymer with eight unsubstituted amino groups. Other examples of core structures include Tris (aminoethyl) amine and

1,2,4,5 benzene tetracarboxylic acid. Each polypeptide is linked to the core structure. Preferably, a cysteine-terminated peptide is linked to thiol-reactive core structure.

In a further aspect, the invention provides chimaeric polypeptides in which a polypeptide of the invention is inserted in or substituted for sequences not essential to the overall architecture or folding pathway of a host protein.

In one alternative the host protein contains one or more SCR repeat, such as an SCR-containing protein of the complement control protein family, for example factor H, C4 binding protein, decay accelerating factor, membrane cofactor protein or complement receptor 2. Such insertions or additions may be used as a a means of adding and/or enhancing anti-complement activity of the host protein. Preferably such substitutions or insertions are made into loop regions (predicted from secondary structure prediction algorithms, homology modelling of tertiary structure or by sequence alignments which identify variable-length insertions in an otherwise conserved sequence background) of the SCR-type module.

In another alternative the host protein is a plasma protein and the insertion or substitution may be used to confer anti-complement activity on the host protein and to alter the stability or pharmacokinetic behaviour of the inserted polypeptide *in vivo*. Suitable examples of such substitutions or insertions include those into a surface loop of an immunoglobulin Fc domain, a non-complementarity-determining region (CDR) of an Fab domain, a turn region of a kringle or growth factor domain or a beta-turn in a 'finger' domain such as those found in fibronectin.

The term 'polypeptide of the invention' will be used hereafter to refer to polypeptides derived from the sequence of general formula (I) as well as multimerised polypeptides and chimeric polypeptides of the invention.

In a further aspect, the invention provides a process for preparing a polypeptide according to the invention which process comprises expressing DNA encoding said polypeptide in a recombinant host cell and recovering the product, and thereafter optionally chemically linking the polypeptide to a core structure.

In particular, the process may comprise the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said polypeptide;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said polypeptide; and

iv) recovering said polypeptide.

The DNA polymer comprising a nucleotide sequence that encodes the polypeptide also forms part of the invention.

The process of the invention may be performed by conventional recombinant techniques such as described in Sambrook *et al.*, Molecular Cloning : A laboratory manual 2nd Edition. Cold Spring Harbor Laboratory Press (1989) and DNA Cloning vols I, II and III (D. M. Glover ed., IRL Press Ltd).

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts *et al.*, in Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical synthesis, by enzymatic polymerisation, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase 1 (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less.

Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to 37°C, generally in a volume of 50µl or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J.Gait, H.W.D. Matthes M. Singh, B.S. Sproat and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771; M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801. Preferably an automated DNA synthesiser (for example, Applied Biosystems 381A Synthesiser) is employed.

The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the polypeptide.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the polypeptide is a routine matter for the skilled worker in the art.

In particular, consideration may be given to the codon usage of the particular host cell. The codons may be optimised for high level expression in *E. coli* using the principles set out in Devereux *et al.*, (1984) Nucl. Acid Res., **12**, 387.

The expression of the DNA polymer encoding the polypeptide in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the polypeptide, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired. The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli,* or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary, fungi e.g. filamentous fungi or unicellular 'yeast' or an insect cell such as Drosophila. The host cell may also be in a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses or vaccinia.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the fragment e.g. bovine papillomavirus vectors in mouse C127 cells, or amplified vectors in chinese hamster ovary cells (DNA Cloning Vol. II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. *et al.*. Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H. Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*,European Patent Application No. 0093619, 1983).

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation

of the DNA, by procedures described in, for example, Sambrook *et al.*,cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Sambrook *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E.coli*, may be treated with a solution of $CaCl_2$ (Cohen *et al.*,Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl, $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol or by electroporation as for example described by Bio-Rad Laboratories, Richmond, California, USA, manufacturers of an electroporator. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells or by using cationic liposomes.

The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Sambrook *et al.*, and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C.

The protein product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial such as *E. coli* and the protein is expressed intracellularly, it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product is usually isolated from the nutrient medium.

Where the host cell is bacterial, such as *E. coli*, the product obtained from the culture may require folding for optimum functional activity. This is most likely if the protein is expressed as inclusion bodies. There are a number of aspects of the isolation and folding process that are regarded as important. In particular, the polypeptide is preferably partially purified before folding, in order to minimise formation of aggregates with contaminating proteins and minimise misfolding of the polypeptide. Thus, the removal of contaminating *E. coli* proteins by specifically isolating the inclusion bodies and the subsequent additional purification prior to folding are important aspects of the procedure.

The folding process is carried out in such a way as to minimise aggregation of intermediate-folded states of the polypeptide. Thus, careful consideration needs to be given to, among others, the salt type and concentration, temperature, protein concentration, redox buffer concentrations and duration of folding. The exact condition for any given polypeptide generally cannot be predicted and must be determined by experiment.

There are numerous methods available for the folding of proteins from inclusion bodies and these are known to the skilled worker in this field. The methods generally involve breaking all the disulphide bonds in the inclusion body, for example with 50mM 2-mercaptoethanol, in the presence of a high concentration of denaturant such as 8M urea or 6M guanidine hydrochloride. The next step is to remove these agents to allow folding of the proteins to occur. Formation of the disulphide bridges requires an oxidising environment and this may be provided in a number of ways, for example by air, or by incorporating a suitable redox system, for example a mixture of reduced and oxidised glutathione.

Preferably, the inclusion body is solubilised using 8M urea, in the presence of mercaptoethanol, and protein is folded, after initial removal of contaminating proteins, by addition of cold buffer. A preferred buffer is 20mM ethanolamine containing 1mM reduced glutathione and 0.5mM oxidised glutathione. The folding is preferably carried out at a temperature in the range 1 to 5°C over a period of 1 to 4 days.

If any precipitation or aggregation is observed, the aggregated protein can be removed in a number of ways, for example by centrifugation or by treatment with precipitants such as ammonium sulphate. Where either of these procedures are adopted, monomeric polypeptide is the major soluble product.

If the bacterial cell secretes the protein, folding is not usually necessary.

Alternatively the polypeptide may be synthesised by conventional solid phase peptide synthesis, for example using an automated peptide synthesiser and Fmoc (9-fluorenylmethoxycarbonyl) chemistry on *para*-alkoxybenzyl alcohol (Wang) resin with the C-terminal amino acid pre-attached.

Accordingly, in a further aspect the invention provides a process for preparing a polypeptide of the invention which comprises condensing appropriate peptide units, and thereafter optionally chemically linking the polypeptide to a core structure.

In the multimeric polypeptide of the invention the polypeptides are preferably linked to the core peptide or multifunctional molecule by way of chemical bridging groups include those described in EP0109653 and EP0152736. The bridging group is generally of the formula:

-A-R-B- (II)

in which each of A and B, which may be the same or different, represents -CO-, $-C(=NH_2^+)-$, maleimido, -S- or a bond and R is a bond or a linking group containing one or more $-(CH_2)-$ or meta- or para- disubstituted phenyl units.

Where the polypeptide and core peptide or multifunctional molecule both include a cysteine the chemical bridging group will take the form -S-S-. The bridge is generated by conventional disulphide exchange chemistry, by activating a thiol on the polypeptide and reacting the activated thiol with a free thiol on the core structure. Alternatively, the free thiol may be on the polypeptide and the activated group on the core structure. Such activation procedures make use of disulphides which generate stable thiolate anions upon cleavage of the S-S linkage and include reagents such as 2,2' dithiopyridine and 5,5'-dithio(2-nitrobenzoic acid, DTNB) which form intermediate mixed disulphides capable of further reaction with thiols to give stable disulphide linkages.

R may include moieties which interact with water to maintain the water solubility of the linkage and suitable moieties include -CO-NH-, -CO-NMe-, -S-S-, $-CH(OH)-$, $-SO_2-$, $-CO_2-$, $-(CH_2CH_2-O)_m-$ and -CH(COOH)- where m is an integer of 2 or more.

Examples of R include $-(CH_2)_r-$, $-(CH_2)_p-S-S-(CH_2)_q-$ and $-(CH_2)_p-CH(OH)-CH(OH)-(CH_2)_q-$, in which r is an integer of at least 2, preferably at least 4 and p and q are independently integers of at least 2.

The bridging group of formula (II) may be derived from a linking agent of formula (III):

$$X-R_1-Y \qquad \text{(III)}$$

in which $R_1$ is a linking group containing one or more $-(CH_2)-$ units and X and Y are functional groups reactable with surface amino acid groups, preferably a lysine or cysteine group, or the N-terminal amino group, or a protein attachment group.

Preferred agents are those where X and Y are different, known as heterobifunctional agents. Each end of the agent molecule is reacted in turn with each molecule to be linked in separate reactions. Examples of heterobifunctional agents of formula (III) include:

3-(2-pyridyldithio) propionic acid N-oxysuccinimide ester

4-(N-maleimido) caproic acid N-oxysuccinimide ester

3-(2-pyridyl) methyl propionimidate hydrochloride

In each case Y is capable of reacting with a thiol group on a polypeptide, which may be a native thiol or one introduced as a protein attachment group.

The protein attachment group is a functionality derived by modification of a polypeptide with a reagent specific for one or more amino acid side chains, and which

contains a group capable of reacting with a cleavable section on the other molecule. An example of a protein attachment group is a thiol group. An example of a cleavable section is a disulphide bond. Alternatively the cleavable section may comprise an $\alpha$, $\beta$ dihydroxy function.

As an example, the introduction of a free thiol function by reaction of a polypeptide or core structure with 2-iminothiolane, 3-(2-pyridyldithio) propionic acid N-oxysuccinimide ester (with subsequent reduction) or N-acetyl homocysteine thiolactone will permit coupling of the protein attachment group with a thiol-reactive B structure. Alternatively, the protein attachment group can contain a thiol-reactive entity such as the 6-maleimidohexyl group or a 2-pyridyl-dithio group which can react with a free thiol in X. Preferably, the protein attachment group is derived from protein modifying agents such as 2-iminothiolane that react with lysine $\varepsilon$-amino groups in proteins.

When X represents a group capable of reacting directly with the amino acid side chain of a protein, it is preferably an N-oxysuccinimidyl group. When X represents a group capable of reacting with a protein attachment group, it is preferably a pyridylthio group.

In the above processes, modification of a polypeptide to introduce a protein attachment group is preferably carried out in aqueous buffered media at a pH between 3.0 and 9.0 depending on the reagent used. For a preferred reagent, 2-iminothiolane, the pH is preferably 6.5-8.5. The concentration of polypeptide is preferably high (> 10mg/ml) and the modifying reagent is used in a moderate (1.1- to 5-fold) molar excess, depending on the reactivity of the reagent. The temperature and duration of reaction are preferably in the range $0^{o}$-$40^{o}$C and 10 minutes to 7 days. The extent of modification of the polypeptide may be determined by assaying for attachment groups introduced.

Such assays may be standard protein chemical techniques such as titration with 5,5'-dithiobis-(2-nitrobenzoic acid). Preferably, 0.5-3.0 moles of protein attachment group will be introduced on average per mole of polypeptide. The modified polypeptide may be separated from excess modifying agents by standard techniques such as dialysis, ultrafiltration, gel filtration and solvent or salt precipitation. The intermediate material may be stored in frozen solution or lyophilised.

Where a protein attachment group is introduced in this way, the bridging group (II) will be formed from a reaction of the linking agent (III) and the protein attachment group.

The polypeptide and core structure to be linked are reacted separately with the linking agent or the reagent for introducing a protein attachment group by typically

adding an excess of the reagent to the polypeptide, usually in a neutral or moderately alkaline buffer, and after reaction removing low molecular weight materials by gel filtration or dialysis. The precise conditions of pH, temperature, buffer and reaction time will depend on the nature of the reagent used and the polypeptide to be modified. The polypeptide linkage reaction is preferably carried out by mixing the modified polypeptide and core structure in neutral buffer at a molar excess of polypeptide appropriate to the number of reactive functionalities in the core structure. Other reaction conditions e.g. time and temperature, should be chosen to obtain the desired degree of linkage. If thiol exchange reactions are involved, the reaction should preferably be carried out under an atmosphere of nitrogen. Preferably, UV-active products are produced (eg from the release of pyridine 2-thione from 2-pyridyl dithio derivatives) so that coupling can be monitored.

After the linkage reaction, the multimeric polypeptide can be isolated by a number of chromatographic procedures such as gel filtration, ion-exchange chromatography, affinity chromatography or hydrophobic interaction chromatography. These procedures my be either low pressure or high performance variants.

The multimeric polypeptide may be characterised by a number of techniques including low pressure or high performance gel filtration, SDS polyacrylamide gel electrophoresis or isoelectric focussing and mass spectrometry.

The polypeptide of this invention is useful in the treatment or diagnosis of many complement-mediated or complement-related diseases and disorders including, but not limited to, those listed below.

**Disease and Disorders Involving Complement**

**Neurological Disorders**
multiple sclerosis
stroke
Guillain Barré Syndrome
traumatic brain injury
Parkinson's disease
allergic encephalitis
Alzheimer's disease

**Disorders of Inappropriate or Undesirable Complement Activation**

haemodialysis complications
hyperacute allograft rejection
xenograft rejection
corneal graft rejection
interleukin-2 induced toxicity during IL-2 therapy
paroxysmal nocturnal haemoglobinuria

**Inflammatory Disorders**

inflammation of autoimmune diseases
Crohn's Disease
adult respiratory distress syndrome
thermal injury including burns or frostbite
uveitis
psoriasis
asthma
acute pancreatitis
vascular inflammatory diseases such as Kawasaki's disease

**Post-Ischemic Reperfusion Conditions**

myocardial infarction
balloon angioplasty
atherosclerosis (cholesterol-induced) & restenosis
hypertension
post-pump syndrome in cardiopulmonary bypass or renal haemodialysis
renal ischemia
intestinal ischaemia

**Immune Complex Disorders and Autoimmune Diseases**

rheumatoid arthritis
systemic lupus erythematosus (SLE)
SLE nephritis
proliferative nephritis
glomerulonephritis
haemolytic anemia
myasthenia gravis

**Infectious Diseases or Sepsis**
multiple organ failure
septic shock

**Reproductive Disorders**
antibody- or complement-mediated infertility

**Wound Healing and Prevention of Scar Formation**

The present invention is also directed to a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide of the invention, as above defined, and a pharmaceutically acceptable carrier or excipient.

The invention also provides a polypeptide of the invention for use as an active therapeutic substance and for use in the treatment of a disease or disorder associated with inflammation or inappropriate complement activation.

The present invention also provides a method of treating a disease or disorder associated with inflammation or inappropriate complement activation comprising administering to a subject in need of such treatment a therapeutically effective amount of a polypeptide of the invention.

In the above methods, the subject is a human or non-human mammal, preferably a human.

An effective amount of the polypeptide for the treatment of a disease or disorder is in the dose range of 0.01-100mg/kg; preferably 0.1mg-10mg/kg.

For administration, the polypeptide should be formulated into an appropriate pharmaceutical or therapeutic composition. Such a composition typically contains a therapeutically active amount of the polypeptide and a pharmaceutically acceptable excipient or carrier such as saline, buffered saline, dextrose, or water. Compositions may also comprise specific stabilising agents such as sugars, including mannose and mannitol, and local anaesthetics for injectable compositions, including, for example, lidocaine.

Further provided is the use of a polypeptide of the invention in the manufacture of a medicament for the treatment of a disease or disorder associated with inflammation or inappropriate complement activation.

The present invention also provides a method for treating a thrombotic condition, in particular acute myocardial infarction, in a subject in need of such treatment. This method comprises administering to a subject in need of this treatment an effective amount of a polypeptide of the invention and an effective amount of a

thrombolytic agent. Such methods and uses may be carried out as described in WO 91/05047.

This invention further provides a method for treating adult respiratory distress syndrome (ARDS) in a subject in need of such treatment, comprising administering to the patient an effective amount of a polypeptide of the invention.

The invention also provides a method of delaying hyperacute allograft or hyperacute xenograft rejection in a subject in need of such treatment which receives a transplant by administering an effective amount of a polypeptide of the invention. Such administration may be to the patient or by application to the transplant prior to implantation.

The invention yet further provides a method of treating wounds in a subject in need of such treatment by administering by either topical or parenteral e.g. intravenous routes, an effective amount of a polypeptide of the invention.

The invention still further provides a method of treating Alzheimer's Disease by administering to a subject in need of such treatment an effective amount of a polypeptide of the invention.

This invention also provides a method of treating CNS inflammatory disorders such as those associated with ischaemic stroke by administering to a subject in need of such treatment an effective amount of a polypeptide of the invention.

## METHODS

### SDS Polyacrylamide gel electrophoresis

Novex precast gels 4-20% were purchased from British Biotechnology and used in Xcell II electrophoresis cells according to the manufacturers instructions.

### Peptide Synthesis

Peptides were synthesised by the solid phase technique using an Applied Biosystems 430A peptide synthesiser and Fmoc (9-fluorenylmethoxycarbonyl) chemistry on *para*-alkoxybenzyl alcohol (Wang) resin with the C-terminal amino acid pre-attached. The resin was treated with benzoic anhydride ( 2 mmol) in the presence of N,N - dicyclohexylcarbodiimide (1 mmol) and 4-dimethylaminopyridine (0.04 mmol) in N-methylpyrrolidone (NMP) and N,N-dimethylformamide (DMF) in order to block any residual free hydroxy groups prior to chain elongation. Each single-coupling cycle consisted of the following steps: 1. The resin was washed with NMP (x1); 2. Fmoc deprotection was carried out with two consecutive treatments (3 min and 15 min) of the resin using a solution of piperidine in NMP (starting concentration 20% v/v); 3. The resin was washed with NMP (x5); 4. The resin was coupled (60 min) with a solution of the preactivated amino acid (1 mmol) in NMP

and DMF; 5. The resin was washed with NMP (x7). In the case of a double-coupling cycle, steps 4 and 5 were conducted twice. Fmoc amino acids (1 mmol) were pre-activated with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU) (1 mmol) in the presence of 1-hydroxybenzotriazole (HOBt) (1 mmol) and N,N-diisopropylethylamine (DIEA) (2 mmol) for 6 to 12 min. After chain elongation, the Fmoc group was removed. The side chain protection used was 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) for arginine, trityl for asparagine, glutamine and cysteine, *tert*-butyloxycarbonyl for lysine and tryptophan, and *tert*-butyl for serine, threonine, aspartic acid and glutamic acid. All residues were double-coupled unless stated.

**Cleavage from the resin**

The ice-cooled peptidyl resin was treated with ice-cooled cleavage mixture A or B (10 ml) and stirred for 2 h at room temperature. The mixture was filtered and the filtrate evaporated *in vacuo* to a low volume (3 to 5 ml) of solution. This was azeotroped *in vacuo* with dry toluene (x 2) and the residual oil triturated with dry diethyl ether (3 x 50 ml) to give a white precipitate. This was collected and dried *in vacuo* to remove any trace of diethyl ether prior to lyophilisation from dilute aqueous acetic acid. The cleavage mixtures used were A: TFA/water/thioanisole/1,2-ethanedithiol (EDT)/phenol (88.9 : 4.4 : 4.4 : 2.2 : 6.7 v/v/v/v/w); B: TFA/water/EDT (75 : 5 : 20 v/v/v).

**High Performance Liquid Chromatography (HPLC)**

Separations were carried out using a Gilson gradient system with detection at 220 nm. Analytical HPLC was conducted on a Spherisorb C-18 column (25 cm x 4.6 mm id) eluted at 1 ml/min and preparative HPLC was conducted on a Spherisorb C-8 column (25 cm x 10 mm id) eluted at 4 ml/min unless stated, with eluents A = 0.1 % aqueous TFA and B = acetonitrile. Gradients used were A: isocratic elution for 5 min at 10% B followed by a 45 min linear gradient to 60% B; B: isocratic elution for 5 min at 10% B followed by a 45 min linear gradeint to 80% B; C: isocratic elution for 5 min at 10% B followed by a 50 min linear gradient to 50% B; D: isocratic elution for 1 min at 10% B followed by a 30 min linear gradient to 80% B; E: isocratic elution for 5 min at 15% B followed by a 60 min linear gradient to 30% B; F: isocratic elution for 1 min at 30% B followed by a 30 min linear gradient to 40% B; G: isocratic elution for 5 min at 10% B followed by a 60 min linear gradient to 40% B; H: isocratic elution for 5 min at 1% B followed by a 60 min linear gradient to 35% B; I: isocratic elution for 5 min at 5% B followed by a 60 min linear gradient

to 30% B; J: isocratic elution for 1 min at 20% B followed by a 30 min linear gradient to 30% B.

**EXAMPLES**

Numbering of peptide residues corresponds to that of human CD35, (C3b/C4b receptor, CR1) (Klickstein *et al.*, 1987, J. Exp. Med. 165:1095-1112; Klickstein *et al.*, 1988, J. Exp. Med 168:1699-1717; Hourcade *et al.*, 1988, J. Exp. Med. 168: 1255-1270). By this numbering, SCR3 of LHR-A is R122-K196:

```
Arg Ile Pro Cys Gly Leu Pro Pro Thr Ile Thr Asn Gly Asp Phe
122                             130

Ile Ser Thr Asn Arg Glu Asn Phe His Tyr Gly Ser Val Val Thr Tyr
            140                                         150

Arg Cys Asn Pro Gly Ser Gly Gly Arg Lys Val Phe Glu Leu Val Gly
                                160

Glu Pro Ser Ile Tyr Cys Thr Ser Asn Asp Asp Gln Val Gly Ile Trp
    170                                         180

Ser Gly Pro Ala Pro Gln Cys Ile Ile Pro Asn Lys
                    190                     196
```

Peptide sequences are presented conventially with N terminal residues to the left.

**EXAMPLE 1: C154-C174 (E1a linear peptide (SEQ ID NO:1), E1b cyclic peptide (SEQ ID NO:2))**

CNPGSGGRKVFELVGEPSIYC (E1)

E1 contains sequence which spans residues C154 - C174 of mature human CR1 which correspond to the second and third cysteines of SCR3. These two cysteines do not normally form a disulphide in wild type CR1 as the C154 pairs with C191 and C174 with the C125.

**1a Synthesis of E1**

Stepwise assembly from Fmoc-Cys(Trt)-resin (0.20 g; 0.10 mmol) gave the 21-residue peptidyl resin with the N-terminal Fmoc group removed (0.57 g). The peptidyl resin (0.28 g) was cleaved using mixture A to give crude solid (0.14 g) after

lyophilisation. The crude product was purified by gel filtration over Sephadex G25 (column 83 cm x 2.5 cm id; detection at 220 nm) using 1M aqueous acetic acid as eluent. The peptide eluted as a single peak which was split into six fractions (combined weight 0.082 g; 49 %): A (11 mg), B (22 mg), C (17 mg), D (24 mg), E (4 mg) and F (4 mg).

**1b Characterisation of E1**

HPLC analysis using gradient F showed the presence of three peaks of retention times 17.8 min (peak 1), 18.6 min (peak 2) and 19.8 min (peak 3) in each fraction in proportions as shown:-

| Fraction | Peak 1 | Peak 2 | Peak 3 | Earlier-eluting material |
|---|---|---|---|---|
| A | 6 | 15 | 61 | 18 |
| B | 15 | 11 | 61 | 13 |
| C | 36 | 14 | 50 | - |
| D | 49 | 16 | 35 | - |
| E | 72 | 20 | 8 | - |
| F | 54 | 14 | 6 | 26 |

Peaks 1 and 3 were shown to be a reduced and oxidised form of the peptide respectively by treatment with dithiothreitol (DTT) and with dimethylsulphoxide (DMSO). Peak 2 was an unknown contaminant which was not affected by DTT nor DMSO. An aqueous solution of fraction B (pH 7.5) was treated with excess DTT; HPLC after 6 h using gradient F showed peak 1 increased whilst peak 3 decreased. Fraction B was treated with an aqueous solution of DMSO (20% v/v); HPLC using gradient F after 11 h showed the disappearance of peak 1 whilst peak 3 increased. An aqueous solution of fraction E (pH 7.5) was treated with excess DTT; HPLC after 5.8 h using gradient F showed the disappearance of peak 3 whilst peak 1 increased. Fraction E was treated with an aqueous solution of DMSO (20% v/v); HPLC using gradient F after 12 h showed the disappearance of peak 1 whilst peak 3 increased.

Electrospray mass spectrometry gave evidence that peaks 1 and 3 were the linear (E1a) and cyclic (E1b) forms of the peptide respectively.

Fraction A gave ions corresponding to $[M+2H]^{2+}$ at m/z 1105.8 (rel. intensity 53%, deconvoluted corresponds to mw 2209.6, calculated for cyclic form 2209.0), m/z 1106.3 (66%, 2210.6), and m/z 1106.8 (53%, 2211.6, calculated for linear form 2210.0).

Fraction C gave ions corresponding to $[M+2H]^{2+}$ at m/z 1105.8 (41%, 2209.6), m/z 1106.4 (66%, 2210.8), m/z 1106.9 (100%, 2211.8), m/z 1107.3 (98%, 2212.6), and m/z 1107.8 (75%, 2213.6).

Fraction F gave ions corresponding to $[M+2H]^{2+}$ at m/z 1106.8 (98%, 2210.6), m/z 1107.3 (99%,2212.6) and m/z 1107.7 (83%,2213.4).

Amino acid analysis: Fraction A: Asx 1.0 (theoretical 1), Glu 2.4 (2), Ser 2.3 (2), Gly 4.2 (4), Arg 1.3 (1), Pro 2.2 (2), Tyr 0.9 (1), Val 1.5 (2), Cys 1.1 (2), Ile 1.1 (1), Leu 1.3 (1), Phe 0.1 (1), Lys 1.7 (1). Fraction C: Asx 1.1, Glu 2.5, Ser 2.1, Gly 4.0, Arg 1.2, Pro 2.1, Tyr 1.0, Val 1.6, Cys 1.1, Ile 1.1, Leu 1.3, Phe 0.1, Lys 1.8. Fraction F: Asx 1.1, Glu 2.4, Ser 2.3, Gly 4.1, Arg 1.2, Pro 2.4, Tyr 1.1, Val 1.4, Cys 0.9, Ile 1.3, Leu 1.1, Phe 0.1, Lys 1.6. (Note: Cys partially destroyed and Val-Phe bond only partially hydrolysed on acid hydrolysis.)

## EXAMPLE 2: S158-C174 (E2 ,SEQ ID NO:3)

SGGRKVFELVGEPSIYC (E2)

This peptide spans the sequence from mature human CR1 S158 to C174.

### 2a Synthesis of E2

Stepwise assembly from Fmoc-Cys(Trt)-resin (0.49 g; 0.25 mmol) gave the 17-residue peptidyl resin with the N-terminal Fmoc group removed (1.03 g). Residues $Ser^{1}$, $Gly^{2,11}$, $Phe^{7}$, $Glu^{8,12}$ and $Pro^{13}$ were single-coupled. The peptidyl resin (0.51 g) was cleaved using mixture A to give crude solid (0.22 g) after lyophilisation. Purification of 0.072 g by preparative HPLC using gradients A, B and C gave purified solid (0.048 g; 66%).

### 2b Characterisation of E2

The product was >95% pure by analytical HPLC and had a retention time of 18.6 min using gradient D. Its identity was verified by observation of a $[M+H]^{+}$ ion in the FAB mass spectrum at m/z 1842 and by an amino acid analysis of Glx 1.97 (theoretical 2), Ser 1.86 (2), Gly 2.85 (3), Arg 1.13 (1), Pro 1.08 (1), Tyr 0.94 (1), Val 1.82 (2), Ile 0.99 (1), Leu 1.12 (1), Phe 1.11 (1), Lys 1.14 (1). (Cys not calculated due to its destruction on acid hydolysis.)

## EXAMPLE 3: Multiple Antigen Peptide (MAP)-E2 conjugate (E3)

To potentiate the activity of S158-C174 (E2), multiple binding sites were created by crosslinking E2 to a lysine core residue.

**3a Derivatisation of MAP peptide.**

(i) N-(2-Pyridyl)dithiopropionyl MAP

MAP peptide (structure $(Lys)_4$ $(Lys)_2$ Ala -OH) was purchased from Peptide and Protein Research, Exeter, UK. Peptide (9.8 mg, 10 micromoles) was dissolved in a mixture of dry dimethylsulphoxide (DMSO,100 microlitres) and dry ACS-grade pyridine (200 microlitres) in which had been dissolved 3-(2-pyridyl)dithiopropionic acid N-oxysuccinimide ester (Pharmacia,25mg, 80 micromoles, 1 mol equivalent to free amino groups in the MAP peptide). The clear solution was agitated gently overnight (15h) at ambient temperature (~22°C) and then stored at -80°C.

(ii) Conjugation to E2

Peptide E2 (as above,7.4mg, 4 micromoles) was dissolved in a mixture of dry DMSO (180 microlitres) and dry ACS-grade pyridine (90 microlitres) and the above PDP-MAP (15 microlitres of solution, ~0.5 micromoles, ~4 micromoles PDP-equivalent) added. The mixture was agitated under dry nitrogen for 6h at ambient temperature and a slight yellow colour was noted. It was then diluted to a final volume of 1.5ml with 20mM Ammonium Bicarbonate pH 7.4 at 4°C. The slightly cloudy solution was applied to a 1 x 10cm column of Sephadex G-25m equilibrated and eluted with the ammonium bicarbonate buffer at 4°C. Fractions eluting between 2.5 and 5.5 ml ,5.5 and 7.5ml and 7.5 and 9.0 ml were collected and lyophilised. Only the first of these contained measurable solid as a white powder (approx 14 mg). .

**3b Characterisation of Map-E2 conjugate**

The elution position of the conjugate on the Sephadex G-25 column suggested an effective molecular weight of ~10,000. This corresponds to a minimum of 4 E2 units disulphide-linked to the MAP (theoretical $M_r$ 9910). The maximum substition is 8 units/MAP (theoretical $M_r$ 17,750).

**EXAMPLE 4: C-(G159-F164)-C (E4, SEQ ID NO:4)**

CGGRKVFC (E4)

This sequence spans residues of G159 - F164 of mature human CR1. To enable circularisation cysteine has been added to the N and C-terminal ends of the peptide.

**4a Synthesis of E4**

Stepwise assembly from Fmoc-Cys(Trt)-resin (0.49 g; 0.25 mmol) gave the 8-residue peptidyl resin with the N-terminal Fmoc group removed (0.74 g). Residues $Gly^{2,3}$ were single-coupled. The peptidyl resin (0.68 g) was cleaved using mixture A to give crude solid (0.22 g) after lyophilisation. Purification by preparative HPLC using gradients G, H and I gave purified solid (0.017 g; 8.6%).

**4b Characterisation of E4**

The product was >90% pure by analytical HPLC and had a retention time of 14.6 min using gradient J. The product was shown to be in an oxidised form by treatment with DTT and with DMSO. An aqueous solution of the product (pH 7.5) was treated with excess DTT; HPLC after 2.3 h using gradient J showed the peak at RT 14.6 min decreased whilst a new peak at RT 15.0 min appeared. The product was treated with an aqueous solution of DMSO (20% v/v); HPLC using gradient J after 1.3 h showed no change. Its identity as the cyclic peptide was verified by observation of a $[M+H]^+$ ion in the FAB mass spectrum at m/z 868.

**EXAMPLE 5: F164-G186 (C174S) (E5, SEQ ID NO:5)**

FELVGEPSIYSTSNDDQVGIWSG (E5)

This peptide spans the residues F164 - G186 of mature human CR1. C174 has been substituted with serine.

**5a Synthesis of E5**

Stepwise assembly from Fmoc-Gly-resin (0.14 g; 0.10 mmol) gave the 23-residue peptidyl resin with the N-terminal Fmoc group removed (0.51 g). Residues $Phe^1$, $Glu^2$, $Gly^5$, $Pro^7$, $Tyr^{10}$, $Ser^{13,22}$, $Asn^{14}$ and $Asp^{15,16}$ were single-coupled. The peptidyl resin (0.24 g) was cleaved using mixture B to give crude solid (0.14 g) after lyophilisation. Purification by preparative HPLC on a Spherisorb C-18 column (25 cm x 4.6 mm id) using gradient E gave purified solid (0.0039 g; 3.3%).

**5b Characterisation of E5**

The product was >90% pure by analytical HPLC and had a retention time of 12.2 min using gradient F. Its identity was verified by observation of a $[M+H]^+$ ion in the FAB mass spectrum at m/z 2501 and by an amino acid analysis of Asx 2.91 (theoretical 3), Glx 3.01 (3), Ser 3.99 (4), Gly 2.88 (3), Thr 1.07 (1), Pro 1.05 (1),

Tyr 0.87 (1), Val 2.47 (2), Ile 1.75 (2), Leu 0.97 (1), Phe 1.00 (1). (Trp not calculated due to its destruction on acid hydrolysis.)

## BIOLOGICAL ACTIVITY

**Anti-Complement Activity Measured By the Haemolysis of Sheep Erythrocytes**

Functional activity of complement inhibitors was assessed by measuring the inhibition of complement mediated lysis of sheep erythrocytes sensitised with rabbit antibodies (obtained from Diamedix Corporation, Miami, USA). Human serum diluted 1/125 in 0.1 M Hepes pH 7.4/ 0.15 M NaCl buffer was the source of complement and was prepared from a pool of volunteers essentially as described in (Dacie & Lewis, 1975). Briefly, blood was warmed to 37°C for 5 minutes, the clot removed and the remaining serum clarified by centrifugation. The serum fraction was split into small aliquots and stored at -196°C. Aliquots were thawed as required and diluted in the Hepes buffer immediately before use. Where indicated , nitrogen gas or helium gas were bubbled through the buffer for approximately 30 minutes after which the bottle containing the buffer was stoppered.

Inhibition of complement-mediated lysis of sensitised sheep erythrocytes was measured using a standard haemolytic assay using a v-bottom microtitre plate format as follows, essentially as described by Weisman *et al* (1990) Science 249 146-151.

50 ul of a range of concentrations of inhibitor diluted in Hepes buffer were incubated with 50 ul of the 1/125. 100 ul of prewarmed sensitised sheep erythrocytes were added and samples incubated for 1 hour at 37°C in a final reaction volume of 200 ul . Samples were spun at 300g at 4°C for 15 minutes before transferring 150 ul of supernatant to flat bottom microtitre plates and determining the absorption at 410 nm, which reflects the amount of lysis in each test solution. Maximum lysis was determined by incubating serum with erythrocytes in the absence of any inhibitor (E+S) from which the proportion of background lysis had been subtracted (determined by incubating erythrocytes with buffer (E). The background lysis by inhibitor was assessed by incubating inhibitor with erythrocytes (E+I) and then subtracting that from test samples (E+I+S). Inhibition was expressed as a fraction of the total cell lysis such that IH50 represents the concentration of inhibitor required to give 50% inhibition of lysis.

Maximum Lysis: $A_{max} = (E+S) - (E)$

Lysis in presence of inhibitor: $A_o = (E+I+S) - (E+I)$

Amount of inhibition: $IH = \frac{A_{max} - A_o}{A_{max}}$

**Results**

**E1** The peptides from each fraction were resuspended in 0.1 M Hepes pH 7.4/0.15 M NaCl buffer which had been made under $N_2$ to remove oxygen and limit the amount of oxidisation to cyclic peptide. The peptides were assayed directly for anti-complement (anti-haemolytic) activity and the results are given below in Table 1.

| Peptide | Peak 1 % Linear | Peak 2 % Circular | IH50 uM Assay 1 | Assay 2 |
|---|---|---|---|---|
| E1 fraction A | 6 | 61 | 200 | 160 |
| E1 fraction B | 15 | 61 | 600 | |
| E1 fraction C | 36 | 50 | 100 | |
| E1 fraction D | 49 | 35 | 75 | 90 |
| E1 fraction E | 72 | 8 | 40 | |

From the data it can be seen that this peptide demonstrates anti-haemolytic activity where increasing potency is correlated with an increase in the proportion of linear peptide (E1a). In forming the cyclic peptide (E1b), a disulphide is formed between two residues that are not normally paired in the native SCR which may constrain the peptide into an unfavourable structure.

**E2** The peptide was assayed using buffer kept under $N_2$ as described above. Three separate assays were carried out and the results gave a mean IH50 of ~670 uM.

**E3** From SDS-PAGE the Mr of the conjugated peptide was estimated as ~ 8000 Da. The IH50 of the MAP peptide alone was approx. 2000 uM and for unconjugated E2 approx 600 uM. Conjugate E3 gave an IH50 of approximately 13 uM indicating a 46 fold improvement in the activity by multimerising the sites.

**E4** The peptide was resuspended in 0.1 M Hepes pH 7.4/0.15 M NaCl buffer which had been made under $N_2$ to remove oxygen and limit the amount of oxidisation to cyclic peptide. The peptide was found to have an IH50 of approximately 300 uM.

**E5** The peptide was resuspended in 0.1M Hepes pH 7.4/0.15M NaCl. Activity of the peptide was determined as approximately 80 uM.

**Seqence listing**

| | | |
|---|---|---|
| SEQ ID NO:1 | linear | CNPGSGGRKVFELVGEPSIYC (E1a) |
| SEQ ID NO:2 | S-S linked cyclic | CNPGSGGRKVFELVGEPSIYC (E1b) |
| SEQ ID NO:3 | | SGGRKVFELVGEPSIYC (E2) |
| SEQ ID NO:4 | | CGGRKVFC (E4) |
| SEQ ID NO:5 | | FELVGEPSIYSTSNDDQVGIWSG (E5) |

**Claims**

1. A polypeptide comprising a portion of the sequence of the general formula (I):
CNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSG (1)
of 6 to 23 amino acids in length and comprising sequence a) and/or b):
a) GGRKVF
b) FELVGEPSIY

2. A polypeptide according to claim 1, comprising cysteine residues at the C and N termini to provide a molecule capable of forming a cyclic molecule bridged by a disulphide bond.

3. A polypeptide according to claim 1 having chemically reactive amino acids at the N or C-terminal ends optionally further derivatised or derivatisable to provide a route for chemical linkage to other peptides or chemicals.

4. A polypeptide according to claim 3 wherein the terminal amino acid is cysteine and the derivative is S- (2-pyridyl) dithio.

5. A polypeptide according to any preceding claim altered at specific amino acids to remove chemically reactive amino acids.

6. A multimeric polypeptide comprising two or more polypeptides according to any preceding claim, linked to a core structure.

7. A multimeric polypeptide according to claim 6 wherein the core structure is a lysine derivative.

8. A multimeric polypeptide according to claim 7 wherein the core structure is $(lys)_4(lys)_2$ lys ala or Tris (aminoethyl) amine and 1,2,4,5 benzene tetracarboxylic acid.

9. A multimeric polypeptide according to claim 6 or 7 having two to eight polypeptides according to any of claims 1 to 5.

10. A chimaeric polypeptide in which a polypeptide according to any of claims 1 to 5 is inserted in or substituted for sequences not essential to the overall architecture or folding pathway of a host protein.

11. A chimaeric polypeptide according to claim 10 in which the host protein contains one or more SCR repeat.

12. A chimaeric polypeptide according to claim 10 in which the host protein is a plasma protein.

13. A polypeptide according to claim 1 selected from:
linear CNPGSGGRKVFELVGEPSIYC
S-S linked cyclic CNPGSGGRKVFELVGEPSIYC
SGGRKVFELVGEPSIYC
CGGRKVFC
FELVGEPSIYSTSNDDQVGIWSG

14. A multimeric polypeptide according to claim 6 which is $(Lys)_4$ $(Lys)_2$ Ala - OH) linked through N-(ε-thiopropionyl) linkers disulphide bonded to cysteine thiol of the peptide

SGGRKVFELVGEPSIYC

15. A process for preparing a polypeptide according to any preceding claim which comprises condensing appropriate peptide units, and thereafter optionally chemically linking the polypeptide to a core structure.

16. A process for preparing a polypeptide according to any preceding claim which process comprises expressing DNA encoding said polypeptide in a recombinant host cell and recovering the product, and thereafter optionally chemically linking the polypeptide to a core structure.

17. A DNA polymer comprising a nucleotide sequence that encodes the polypeptide according to any of claims 1 to 5 or 10 to 12.

18. A replicable expression vector capable, in a host cell, of expressing the DNA polymer of claim 17.

19. A host cell transformed with a replicable expression vector of claim 18.

20. A pharmaceutical composition comprising a therapeutically effective amount of a polypeptide according to any of claims 1 to 14 and a pharmaceutically acceptable carrier or excipient.

21 A polypeptide according to any of claims 1 to 14 for use as an active therapeutic substance.

22. A polypeptide according to any of claims 1 to 14 for use in the treatment of a disease or disorder associated with inflammation or inappropriate complement activation.

23. A method of treating a disease or disorder associated with inflammation or inappropriate complement activation comprising administering to a subject in need of such treatment a therapeutically effective amount of a polypeptide according to any of claims 1 to 14.

24. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for the treatment of a disease or disorder associated with inflammation or inappropriate complement activation.

25. A method for treating a thrombotic condition in a subject in need of such treatment comprising administering to a human or animal in need of this treatment an effective amount of a polypeptide according to any of claims 1 to 14 and an effective amount of a thrombolytic agent.

26. A method for treating adult respiratory distress syndrome (ARDS) in a subject in need of such treatment, comprising administering to the patient an effective amount of a polypeptide according to any of claims 1 to 14.

27. A method of delaying hyperacute allograft or hyperacute xenograft rejection in a subject in need of such treatment which receives a transplant by administering an effective amount of a polypeptide according to any of claims 1 to 14.

28. A method of treating wounds in a subject in need of such treatment by administering by either topical or parenteral routes, an effective amount of a polypeptide according to any of claims 1 to 14.

29. A method of treating Alzheimer's Disease by administering to a subject in need of such treatment an effective amount of a polypeptide according to any of claims 1 to 14.

30. A method of treating CNS inflammatory disorders by administering to a subject in need of such treatment an effective amount of a polypeptide according to any of claims 1 to 14.

31. The use of a polypeptide according to any of claims 1 to 14 and an effective amount of a thrombolytic agent in the manufacture of a medicament for the treatment of a thrombotic condition.

32. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for the treatment of adult respiratory distress syndrome (ARDS) in a subject in need of such treatment, comprising administering to the patient an effective amount of a polypeptide according to any of claims 1 to 14.

33. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for delaying hyperacute allograft or hyperacute xenograft rejection.

34. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for treating wounds.

35. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for treating Alzheimer's Disease.

36. The use of a polypeptide according to any of claims 1 to 14 in the manufacture of a medicament for treating CNS inflammatory disorders.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP 97/00994

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C12N15/12 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 94 00571 A (SMITHKLINE BEECHAM PLC ;SMITH RICHARD ANTHONY GODWIN (GB); DODD IA) 6 January 1994 cited in the application see the whole document --- | 1-4,6-36 |
| A | EP 0 512 733 A (UNIV WASHINGTON) 11 November 1992 see the whole document --- | 1-4,6-36 |
| A | WO 91 05047 A (UNIV JOHNS HOPKINS ;BRIGHAM & WOMENS HOSPITAL (US); T CELL SCIENCE) 18 April 1991 cited in the application see the whole document --- | 1-4,6-36 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 July 1997 | 07.08.97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016 | Groenendijk, M |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International lication No
PCT/EP 97/00994

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 94 16719 A (SMITHKLINE BEECHAM PLC ;MOSSAKOWSKA DANUTA EWA IRENA (GB); SMITH R) 4 August 1994<br>see the whole document<br>----- | 31 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Intern ıal application No.
PCT/EP 97/00994

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 23, 25-30
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 23, 25-30 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The use of expressions like "altered (to what ?) and "chemically reactive amino acids" (under which conditions ?) in claim 5 renders this claim unclear under Art. 6 PCT. Claims searched completely: 1-4,6-36
Claim not searched: 5

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International lication No
PCT/EP 97/00994

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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(54) Title: FRAGMENTS OF CR1 AND THEIR USE

(57) Abstract

A polypeptide comprising a portion of the sequence of the general formula (I): CNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSG, of 6 to 23 amino acids in length and comprising sequence a) and/or b): a) GGRKVF, b) FELVGEPSIY multimeric and chimaeric derivatives, pharmaceutical compositions containing them and their use in therapy.

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416) |
|---|---|---|
| JMD/JS/39156 | | |
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| C07K14/705 | | |
| Applicant | | |
| ADPROTECH PLC et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 16/09/1997 | 12.05.98 |
| Name and mailing address of the IPEA/ | Authorized officer |
| European Patent Office<br>D-80298 Munich<br>Tel. (+49-89) 2399-0, Tx: 523656 epmu d<br>Fax: (+49-89) 2399-4465 | Döpfer, K-P<br>Telephone No. (+49-89) 2399-8547 |

Form PCT/IPEA/409 (Cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP97/00994

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-24 as originally filed

**Claims, No.:**

1-36 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5,25-30.

because:

☒ the said international application, or the said claims Nos. 23,25-30 relate to the following subject matter which does not require an international preliminary examination (*specify*):

**see separate sheet**

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 5 are so unclear that no meaningful opinion could be formed (*specify*):

**see separate sheet**

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-4,6-22,24,31-36 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-4,6-22,24,31-36 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-4,6-22,24,31-36 |
| | No: | Claims | 23, 25-30 |

2. Citations and explanations

**see separate sheet**

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

Reference is made to the following documents:

D1: WO-A-94/00571
D2: EP-A-512 733
D3: WO-A-91/05047
D4: WO-A-94/16719

1. **Section III**

Present claims 23, 25-30 are directed to a method of treatment of the human or animal body by therapy. This subject-matter does not require international preliminary examination (Rule 67.1 iv) PCT. For the assessment of present claim 5 see point 2 of this report.

2. **Section VIII:**

Dependent claim 5 is directed to "*a polypeptide according to any preceding claim altered at specific amino acids to remove chemically reactive amino acids*". It is not clear which amino acid at which position in the polypeptide has to be altered in which manner to achieve the desired result. Furthermore, the term "chemically reactive amino acids" used has no defined meaning. Accordingly, present claim does not fulfill the requirements of Article 6 PCT. The present claim is so unclear that a meaningful examination is not possible whether the subject-matter of claim 5 is novel, involves an inventive step and is industrial applicable (Article 33 PCT). The same is to be applied to subject-matter of the claims relating to dependent claim 5.

3. **Section V:**

The present application is directed to peptide fragments comprising a portion of the sequence CNPGSGGRKVFELVGEPSIYCTSNDDQVGVGIWSG (I) derived from the complement receptor type 1 (CR1) and their use in diagnosis and therapy.

D1, which is considered representing the closest prior art, discloses soluble CR1 derivatives selected from short consensus repeats (SCR) 1 to 4 of long homologous repeat A (LHR-A) including at least SCR3, with the general formula $H_2N$-$V^1$-SCR1-$W^1$-SCR2-$X^1$-SCR3-$Y^1$-OH (with e.g. $aa_{125-191}$ of mature SCR3) having functional complement inhibitory, including anti-haemolytic, activity.

D2 discloses analogues of regulators of complement activation (RCA) proteins. These analogues are obtained by substituting amino acids which effect the binding of these proteins (amino acids 35, 64-65, 92-94 (C4b) and the sequence STKP(PIC)Q (C3b) in the CR1 protein.

D3 discloses soluble fragments (at least 24 amino acids long, see in particular peptide No. 54d, Table I , page 53) of CR1 which exhibit functional activity, binding C3b and/or C4b and demonstrate Factor I cofactor activity (see claims).

D4 discloses the use of soluble CR1 proteins in combination with amidinophenyl or amidinonaphthyl esters for treating a disease or disorder associated with inflammation or inappropriate complement inactivation.

None of the cited prior art documents discloses the claimed peptides. Accordingly, the subject-matter of present claims 1-4, 6-22, 24 and 31-36 is new over the prior art as cited (Article 33(2) PCT).

The problem underlying the present application can be regarded as to provide further CR1 derived substances for influencing states which are associated with pathological complement activation.

The solution are peptides comprising a portion of the sequence CNPGSGGRKVFELVGEPSIYCTSNDDQVGVGIWSG (I) of 6-23 amino acids in length and comprising sequence (a) GGRKFV and/or (b) FELVGEPSIY, multimeric and chimeric derivatives. The closest prior art does disclose CR1 derived peptides which comprise sequence (b) (see D1; Table I, peptide 54d). These peptide are much longer than the claimed ones. Furthermore, there are no concrete data given for the biological activities of these tryptic digests. The skilled person dealing with the above formulated problem has no guidance from any